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STUDIES ON THE MICROBIOLOGY OF STERILIZED SOIL IN RELATION TO ITS INFESTATION WITH PLANT PATHOGENS

Ralph Antony Ludwig

Department of Field Crops

University of Alberta

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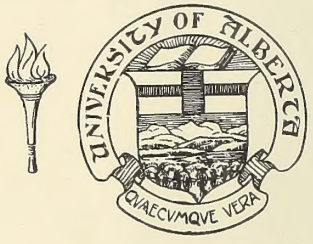

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Ralph Antony Ludwig

Department of Field Crops

A THESIS

submitted to the University of Alberta in

partial fulfilment of the requirements for

the degree of

MASTER OF SCIENCE

This thesis represents one-half of the total work

Edmonton, Alberta

April, 1939

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STUDIES ON THE MICROBIOLOGY OF STERILIZED SOIL IN RELATION TO ITS INFESTATION WITH PLANT PATHOGENS

Ralph Antony Ludwig

INTRODUCTION

The effect of plant pathogens on the underground parts of plants is often greatly modified by the naturally occurring soil microorganisms. This has now been demonstrated (12, 25) to be the case under Edmonton conditions for the foot-rotting pathogens of cereals. Other soil-borne fungi, Rhizoctonia for example, have been found to behave similarly. It has also recently been shown (15) that certain seed-borne pathogens are affected in a like manner. Such an effect is not surprising when it is considered that a gram of Edmonton black loam contains approximately eight million bacteria, four million actinomycetes and fifty thousand fungi. These microorganisms, under natural conditions, are all more or less at equilibrium with one another. A plant pathogen introduced into the soil, on the seed or otherwise, must compete with the naturally occurring saprophytic organisms for its existence. Such pathogens may be weakly saprophytic and only able to become established by growing on a more selective medium, namely the host tissue.

It has often been observed that the "take-all" fungus (Ophiobolus graminis Sacc.), with which this paper is primarily concerned, may cause extensive damage to a wheat crop during one season and comparatively little damage the following season. It has been suggested (12) that this phenomenon may be partly due to a direct effect of the climatic conditions on the pathogen itself and partly to their effect on the soil saprophytes which in turn influence the pathogen. Padwick (20) found that, when either sterilized or unsterilized soil was inoculated with Ophiobolus and then left bare for five to eight weeks before seeding with wheat, little or no "take-all" infection was obtained. Similarly it has been noticed that, even though 100% infection is obtained in a first seeding of a pot experiment, a second seeding will commonly give practically disease-free plants.

Experimental work with the foot- and root-rotting fungi has often necessitated the use of sterilized soil. It has been observed in this laboratory that the behavior of certain plant pathogenic fungi is often different in sterilized recontaminated soil than in natural unsterilized soil. This is particularly true of Ophiobolus graminis.

The object of the present work was to study some of the microbiological changes resulting from sterilization and recontamination* of the soil in an attempt to correlate

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* In this work the term "recontamination" will be used to indicate the introduction of saprophytes into soil. The term "infestation" will be used only in connection with the introduction of the pathogen into soil, and the term "inoculation" with the deposition of the pathogen in contact with the host.

them with the peculiar behavior of Ophiobolus graminis on this medium. It is hoped that, when the conditions surrounding the decline of Ophiobolus in sterilized recontaminated soil are better understood, similar conditions may be induced as a practical control measure for "take-all" in field soils.

THE COMPARATIVE REACTION OF WHEAT SEEDLINGS TO *OPHIOBOLUS GRAMINIS* SACC. IN STERILIZED RECONTAMINATED AND UNSTERILIZED SOIL

Introduction

Differences in the reaction of wheat seedlings to *Ophiobolus graminis* in sterilized and unsterilized soil have been previously studied in this laboratory. Henry (14), for example, studied the influence of soil sterilization and soil temperature on the reaction of wheat seedlings to *Ophiobolus graminis*. In his experiments soil was sterilized for four hours at 15 pounds pressure, after which the pathogen was added and then surface sterilized wheat seed was sown immediately. On examination of the wheat seedlings after three weeks, it was found that at 13°C blighting was equally severe in both sterilized and unsterilized soil. At 27° most of the seedlings were killed in the sterilized soil, while those in the unsterilized soil were only slightly attacked. Later unpublished work by the same author indicated that, when sterilized soil was recontaminated with a little normal soil, less "take-all" damage might occur in it than in the normal soil. With the object of confirming these observations a comparative study of sterilized soil recontaminated with a little normal soil, and natural unsterilized soil was undertaken.

Experimental Methods and Results

The pathogen used in these experiments was the same as that used by Henry (14), namely Ophiobolus IV, a virulent strain of Ophiobolus graminis.

Experiment I.

Methods.

Inoculum was produced on a medium consisting of Edmonton black loam plus 10% cornmeal. This inoculum was produced in 200 cc. Erlenmeyer flasks, each containing 50 grams of the air-dry soil-cornmeal mixture plus 27 cc. of tap water. These flasks were sterilized for two hours at 15 pounds steam pressure and then sown with Ophiobolus from potato dextrose agar slants. The fungus was allowed to develop for three to four weeks at room temperature, by which time the mycelium had completely penetrated the substrate.

Where sterilized soil was used the pots were sterilized for eight hours at 15 pounds pressure. All pots were well watered prior to sterilizing. All pots for this experiment were made up at one time and held in a dry condition in the greenhouse until they were needed. A three to one black loam, sand mixture was used. On the 19th of May, 1938, a series of pots were sterilized, cooled and immediately recontaminated with a little

normal soil. These and a corresponding series of unsterilized pots were then incubated in a moist condition in the greenhouse. On the 6th of June a second set of pots of sterilized and unsterilized soil was prepared and placed to incubate. A final set was prepared on the 4th of July, at which time all the pots were sown. At this time each of the six series was divided into three groups of eight pots each. One was left uninoculated as a check, while the remaining two groups were inoculated with Ophiobolus at the rate of half a flask and one flask of inoculum per pot. Two rates of inoculation were employed since one might produce a more marked difference than the other. This fungus was added to the pots at the seed level. Twenty-five seeds of good quality Red Bobs wheat were used in each pot. All pots were randomized in a block on a single greenhouse bench. During the experiment the temperature of the house was held as close to 20°C. as was possible.

Results.

After a period of three weeks notes were taken on plant height and disease severity. The height of each plant, from the seed to the tip of the longest leaf, was measured to the nearest centimeter. Disease severity was expressed as a percentage. It was calculated by classifying the plants according to a numerical rating from 0 to 5. The resulting score obtained for each pot was then expressed as a percentage of the maximum possible

score. It should be borne in mind that a negative relationship exists between plant height and degree of infection in percent. A high degree of infection should accompany a low plant height. However, where the degree of infection is high, plant height is not a direct index of disease severity. This is a logical observation since under most conditions Ophiobolus does not cause a pre-emergence blight. In the case of 100% infection the height attained by the plants before finally succumbing to the pathogen varies with the conditions of the experiment.

The summarized height data are presented in Table I (A) and the corresponding degree of infection in percent in Table I (B). Differences in height significant beyond the 1% point are shown between the plants grown in sterilized recontaminated soil and those grown in unsterilized soil as well as between those incubated for different periods. The amount of disease, as evidenced by both plant height and degree of infection shows that, for both the shortest and the longest incubation periods, the pathogen was more effectively suppressed in sterilized recontaminated soil than in unsterilized soil. This difference did not hold for the intermediate incubation period. The average plant height for all dates gives a figure for the unsterilized soil which is significantly lower than that for sterilized recontaminated soil. More pronounced differences are evident where the lighter rate of inoculation was used. The data giving the degree of

TABLE I

Comparative reaction of wheat seedlings to Ophiobolus graminis in sterilized recontaminated soil and unsterilized soil

A. Plant height

No. of flasks of inoculum per pot	Days prior to seeding[1]									Average[2]		
	0			28			46					
	Sterilized	Unsterilized	Difference	Sterilized	Unsterilized	Difference	Sterilized	Unsterilized	Difference	Sterilized	Unsterilized	Difference
0	32.7*	28.2	4.5	34.9	30.9	4.0	35.3	33.0	2.3	34.3	30.8	3.5
½	29.7	13.4	16.3	9.5	11.2	-1.7	20.8	8.0	12.8	20.0	10.9	9.1
1	22.0	11.2	10.8	6.9	8.7	-1.8	14.5	5.9	8.6	14.5	8.6	5.9

Minimum significant difference = 5.97

B. Degree of infection in percent

0	0**	3.2	- 3.2	0	1.2	- 1.2	0	1.2	- 1.2	0	1.9	- 1.9
½	15.3	65.9	-50.6	86.0	76.4	9.6	52.7	82.4	-29.7	51.0	74.9	-23.9
1	38.5	71.9	-33.4	92.9	77.0	15.9	69.8	91.4	-21.6	67.6	80.1	-12.5

* Average plant height in centimeters based on 8 pots of 25 seeds each.
** Percentage disease based on an average of 8 pots.
1 Length of incubation period.
2 Average of all dates.

infection, Table I (B), are in agreement with those for height. They cannot, however, be treated statistically (3) since the zero values given in the controls present a false estimate of treatment variability.

Experiment II.

Methods.

The data presented here were obtained from a fertilizer experiment which was conducted in cooperation with the Department of Soils. In this experiment both sterilized recontaminated and unsterilized soils were used. While it was primarily a fertilizer test, its design permitted further comparison of sterilized recontaminated and unsterilized soil.

Treatments in this case consisted of three organic fertilizers, namely, dried green alfalfa, mature wheat straw and poplar sawdust. Each of these was used singly and in combination with an inorganic fertilizer, namely, ammonium phosphate (16-20). The finely ground amendment plus a small amount of unsterilized soil was mixed thoroughly with the soil in the top half of each pot. All pots were then incubated at approximately 20°C. for a period of 25 days. Inoculated series were replicated eight times and the checks only four times. Inoculum was grown on sterilized black soil and, owing to the somewhat poor growth obtained, was supplemented at the time of seeding with 10% of inoculum grown on an oat hull dextrose medium. The

methods of seeding and inoculation were the same as in Experiment I with the exception that 40 grams of the mixed inoculum were used in each pot. All pots were randomized in a block on a single greenhouse bench.

Results.

After a period of four weeks notes were taken on plant height, dry weight of plants per pot and degree of infection in percent. The complete data for this experiment are presented in Appendix I, while the average for all organic and inorganic fertilizer treatments are given in Table II.

Since fewer pots were used in the checks than in the inoculated series, the analysis of variance was applied according to the method given in Snedecor (26) for the treatment of unequal numbers. The variance for the height data is given in Table III.

TABLE III

Variance for height data

Source of variance	D.F.	Mean square	F	1%
Fertilizers	7	175.75	4.03	3.66
Inoculations	3	1,247.50	28.61	4.87
Fertilizers x Inoculations	21	43.60	6.02	1.97
Residual	160	7.24		
Total	191			

TABLE II

Further comparative data on the reaction of wheat seedlings to Ophiobolus graminis, based on averages of a number of fertilizer treatments on sterilized recontaminated and unsterilized soil

Fertilization treatment	Inoculation treatment	Sterilized recontaminated soil			Unsterilized soil			Difference		
		Height	Dry weight	Infection	Height	Dry weight	Infection	Height	Dry weight	Infection
Averages	Check	36.2[1]	1.15[2]	0.2[3]	34.2	1.10	0.8	2.0	0.05	- 0.6
	Inoculated	29.8	0.96	36.5	24.5	0.75	52.7	5.3	0.21	-16.2
Minimum significant difference*		3.29	0.140		3.29	0.140		3.29	0.140	

* Minimum significant difference for inoculations.

1 Average plant height in centimeters.

2 Average dry weight per pot in grams.

3 Degree of infection in percent.

The mean square for the fertilizer x inoculation interaction was used in testing the significance of the inoculation treatments as well as in obtaining the minimum significant difference.

Differences between the dry weight of plants in sterilized recontaminated and unsterilized soil were also significant beyond the 1% point. Data giving the percentage degree of infection were not analyzed. Dry weight and percentage infection data show the same trends as the data for plant height. Since it is the general comparison rather than the amendments with which this paper is concerned, the data for the check series have been analyzed separately and are presented in Table IV.

TABLE IV

Data showing the comparative reaction of wheat seedlings to Ophiobolus graminis on sterilized recontaminated and unsterilized soil*

	Sterilized recontaminated	Unsterilized	Difference	Minimum significant difference
Height	24.9[1]	16.7	8.2	3.29
Dry weight	0.85[2]	0.60	0.25	0.121
% infection	52.9[3]	81.0	-28.1	11.85

[1] Average plant height in centimeters.
[2] Average dry weight per pot in percent.
[3] Degree of infection in percent.

* Based on figures taken from the unfertilized inoculated series in the experiment summarized in Table II.

Data for plant height, dry weight and percentage infection all show differences which are significant beyond the 1% point.

The extent of the difference between unsterilized and sterilized recontaminated soil may, perhaps, be best appreciated by an examination of Figure 1. The inoculated pots shown in this photograph are representatives of the two series from which the data for Table IV were obtained.

Discussion.

From the results given in the previous tables, and from other similar data not presented here, it is concluded that, in general, less severe infection of wheat seedlings by Ophiobolus graminis occurs in Edmonton black soil that has been sterilized and then recontaminated with a little normal soil, than in the normal unsterilized soil. Henry (12, 14) has shown that a certain suppressive action on cereal foot-rot fungi is exerted by the microorganisms of unsterilized soil, an effect which obviously could not occur in uncontaminated sterilized soil. This effect applies to Ophiobolus, Helminthosporium and Fusarium. Thus there is progressively less infection of wheat seedlings by Ophiobolus graminis in sterilized soil, unsterilized soil and sterilized recontaminated soil.

Any theory which is advanced to explain these differences must take into account the fact that a decrease of infection in sterilized recontaminated soil does not

Figure 1

The comparative reaction of wheat seedlings to *Ophiobolus graminis* in sterilized recontaminated and unsterilized soil*

Left to right - Unsterilized soil, unsterilized soil plus *Ophiobolus*, sterilized recontaminated soil plus *Ophiobolus* and sterilized recontaminated soil.

* The unfertilized inoculated series of the experiment summarized in Table II.

always occur. This is evidenced by one of the series shown in Table I as well as by similar discrepancies in other experiments not reported here. If the suppressive effect is due to individual soil organisms or to groups of soil organisms such irregularities as these might be expected since under certain conditions organisms other than those responsible for the effect may predominate.

A number of hypotheses have been advanced to explain such effects. Of these, the one adopted by Henry (12), Sanford and Broadfoot (25) and others appears to be the most plausible. They suggest that the microorganisms naturally occurring in the soil exert a definite antagonistic action on the pathogens. Such an effect may be accomplished by an exhaustion of the food supply, by the excretion of substances which are toxic or lytic to the pathogen, or by the actual parasitism of the plant pathogen by these soil microorganisms.

No direct evidence to prove that, in soil, the food supply is used by the saprophytes to the exclusion of a pathogen, such as Ophiobolus, has been obtained. There is no reason to suppose, however, that such a relationship could not exist. Considerable indirect evidence points to such an effect.* In view of the fact that the main plant foods, particularly nitrogen, tend to be changed to a more soluble form (18, 22, 24), and consequently are made more readily available, ~~by the action of~~ to the microorganisms in partially sterilized and sterilized recontaminated soil,

* That the food supply is used by the saprophytes to the exclusion of the pathogens.

the idea that any of the main food materials are used up appears to be improbable. If any material is used up by the saprophytes to the exclusion of the pathogen, it must be something required only in small amounts. Padwick (21) demonstrated the role of a growth substance for Ophiobolus graminis in artificial culture, and suggested that such a substance might be liberated in soil by the sterilization process. This might account for the difference between unsterilized and sterilized soil, but will not in itself account for the decrease in disease severity in sterilized recontaminated soil. It is possible, however, that such a substance may exist in soil or be liberated into it by heat. Where the soil is sterilized but not recontaminated, the pathogen may be able to take advantage of the growth substance. In the sterilized recontaminated soil, on the other hand, the fast growing saprophytic organisms may be able to utilize all of the available material with the result that the slower growing pathogen cannot benefit from it.

Naturally occurring materials toxic to Ophiobolus have never, as far as the writer knows, been isolated from the soil. Sanford and Broadfoot (25) obtained a toxic filtrate by growing saprophytes in potato decoction. This, when applied to soil inoculated with Ophiobolus, reduced the severity of wheat seedling infection. Similarly, Weindling (34) showed that certain culture extracts of Trichoderma and Gliocladium were toxic at very high dilutions to Rhizoctonia solani.

Definite parasitism of growing fungal hyphae by a strain of Trichoderma lignum has been reported by Weindling (32). The vegetative hyphae of Rhizoctonia solani and Pythium sp., as well as a variety of other fungi, were definitely digested. Drechsler (5) also observed parasitism. He, for example, describes two moniliaceous fungi, Trinacrium subtile and Dactylella spermatophaga, which are vigorously parasitic on the oospores of various species of Pythium.

It seems probable that all of the above-mentioned factors may play a part in the suppression of plant pathogens in the soil. The difficulty of determining their relative importance may be ascribed to the fact that the soil is such a complex growth medium.

COMPARATIVE STUDIES ON THE MICROBIAL POPULATIONS OF STERILIZED RECONTAMINATED AND UNSTERILIZED SOIL

In the preceding section less disease due to Ophiobolus graminis was found to develop in sterilized recontaminated soil than in unsterilized soil. A number of possible explanations were reviewed. The following studies were made in an attempt to explain this difference.

Comparative Changes in Numbers of Microorganisms

Introduction.

The fact that tremendous increases in the number of microorganisms follows partial sterilization of the soil (24, 30, 31) has been known for a long time. Russel and Hutchinson (24) noted from five to nine million bacteria in untreated soil and 40 million in soil that had been partially sterilized. Partial sterilization treatments differ from complete sterilization and recontamination in that, in the latter case, all the organisms originally present are returned after treatment, while in the former only those resistant to the sterilization treatment remain.

Method.

The numbers of bacteria, fungi and actinomycetes were determined by the plate count method. In a preliminary experiment, soil was placed in small flasks and a single flask then used for each plating. This method was unsatisfactory because a separate body of soil was used in each determination and because of the tendency for profuse spore production where the sterilized recontaminated soil came against the sides of the glass flasks. The method finally employed was a modification of this. A sample of soil was obtained from the west field, University of Alberta campus. This was taken at a depth of two to three inches, after removing the surface inch. One thousand grams of the moist soil was placed in each of four 2000 cc. erlenmeyer flasks, the exact moisture content determined and the soil in all the flasks raised to a moisture level of 23%. The flasks were then weighed. A continuous record of the weights was kept for the duration of the experiment, and all flasks were maintained at this moisture level by additions of sterile water. Two of the four flasks were sterilized for four hours at 15 pounds steam pressure, and after cooling were recontaminated with a small amount of the original soil. A sampler was devised which enabled the drawing of samples under sterile conditions. By this method, a core of soil 5/8 of an inch in diameter and 2 to 2½ inches long was removed. It enabled sampling to a uniform depth, and made possible the

avoidance of the extreme edges of the soil mass. One sample from each flask was plated at each time. The sample was dropped immediately into a sterilized and previously weighed water blank in a shaker bottle. The weight of the sample was then determined by the increase in the weight of the bottle. Since relative and not absolute figures were desired, the numbers were computed on the basis of a gram of the moist soil. The plating technique was essentially the same as that devised by Sutherland and James (27) and James (16). A few minor modifications, however, were introduced to adapt the procedure to the equipment available. Czapek's medium (8) with 17.5, instead of 15 grams of agar per liter, was employed for fungi. This medium was poured into flasks, 100 cc. per flask, and acidified at the time of using with ½ cc. of a solution of 10 cc. of 85% lactic acid in 100 cc. of water. One flask of this medium was used to pour ten plates. For bacteria and actinomycetes, sodium albuminate agar (8) was used. A 100 cc. flask of this medium was also used to pour ten plates. The numbers of bacteria and actinomycetes were determined on the same plates. All plates were incubated at 25°C. and the fungal counts made after four days, the bacterial after eight days and the actinomycete after 12 days. Where there was any doubt as to the nature of the colony, a microscopical examination was made.

Results.

The counts were recorded on the basis of numbers per gram of moist soil. Separate records for each flask were kept.

Fungi.

The fungal data are given in Table V and represented graphically in Figure 2. The fungal numbers in the two flasks of unsterilized soil agree quite closely, there being from 20 to 30 thousand per gram.

TABLE V

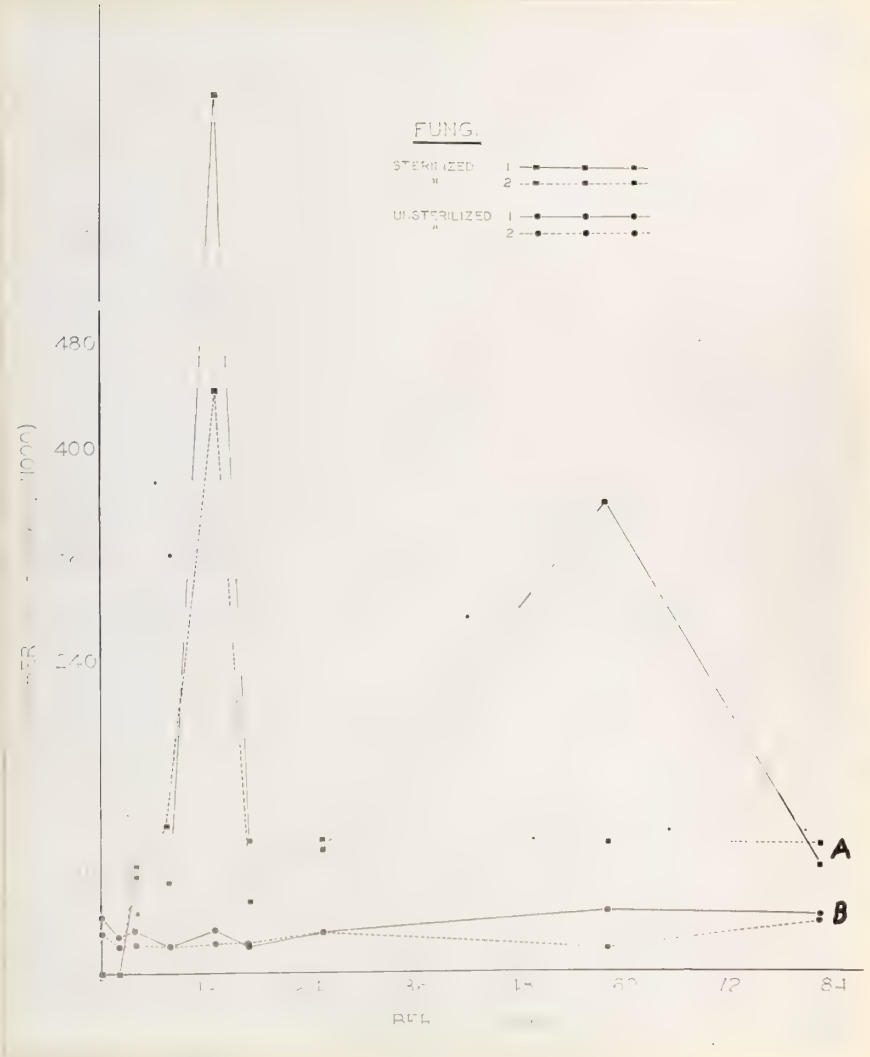
Comparative numbers of fungi in sterilized recontaminated and unsterilized soil

No.of days	Sterilized recontaminated		Unsterilized		Average		
	A	A	B	B	Steri-lized	Unsteri-lized	Differ-ence
0	4	5	29,750	40,500	4	35,125	-35,121
2	80	18	20,700	26,125	- 50	23,412	-23,362
4	80,000	73,500	21,850	30,625	76,750	26,238	50,512
8	110,000	70,000	20,000	20,000	90,000	20,000	70,000
13	441,000	660,000	23,375	30,250	550,500	26,812	523,678
17	55,000	99,000	19,250	17,875	77,000	18,562	58,438
25	98,000	88,000	27,500	27,600	93,000	27,550	65,450
57	99,000	350,000	15,312	45,000	224,500	30,156	194,344
80	92,000	81,675	38,167	40,375	86,838	39,271	67,567

The numbers of organisms in the flasks of sterilized recontaminated soil do not correspond so well. The figures for both flasks, however, show tremendous

Figure 2

Comparative numbers of fungi in sterilized recontaminated and unsterilized soil



A. Sterilized recontaminated soil.

B. Unsterilized soil.

increases over the unsterilized soil. In one instance a count of over 650 thousand was obtained. The rise in numbers, as will be noted, was practically negligible at the two day period, but very great in four days, and at a maximum in 12 to 13 days. After the maximum was reached there was a rapid decline in numbers, but at the end of 80 days the general level was still above that of the unsterilized soil.

Bacteria.

The trend in bacterial numbers (Table VI, Figure 3) is roughly the same as that shown by the fungi. Again, counts on unsterilized soil remained comparatively constant, while those on the sterilized recontaminated soil fluctuated widely. A maximum in eight to 13 days was, however, quite definite in one replicate. The numbers in unsterilized soil ranged from eight to ten million per gram, while in sterilized recontaminated soil as high as 300 million was reached.

Actinomycetes.

No colonies of actinomycetes were obtained in early platings of sterilized recontaminated soil, even though these were abundantly present at the start, Table VII, Figure 4. Colonies began to appear in eight to 13 days, and in one case, one of the two replicates finally became equal in number to those from unsterilized soil. The numbers in the other replicate greatly exceeded those

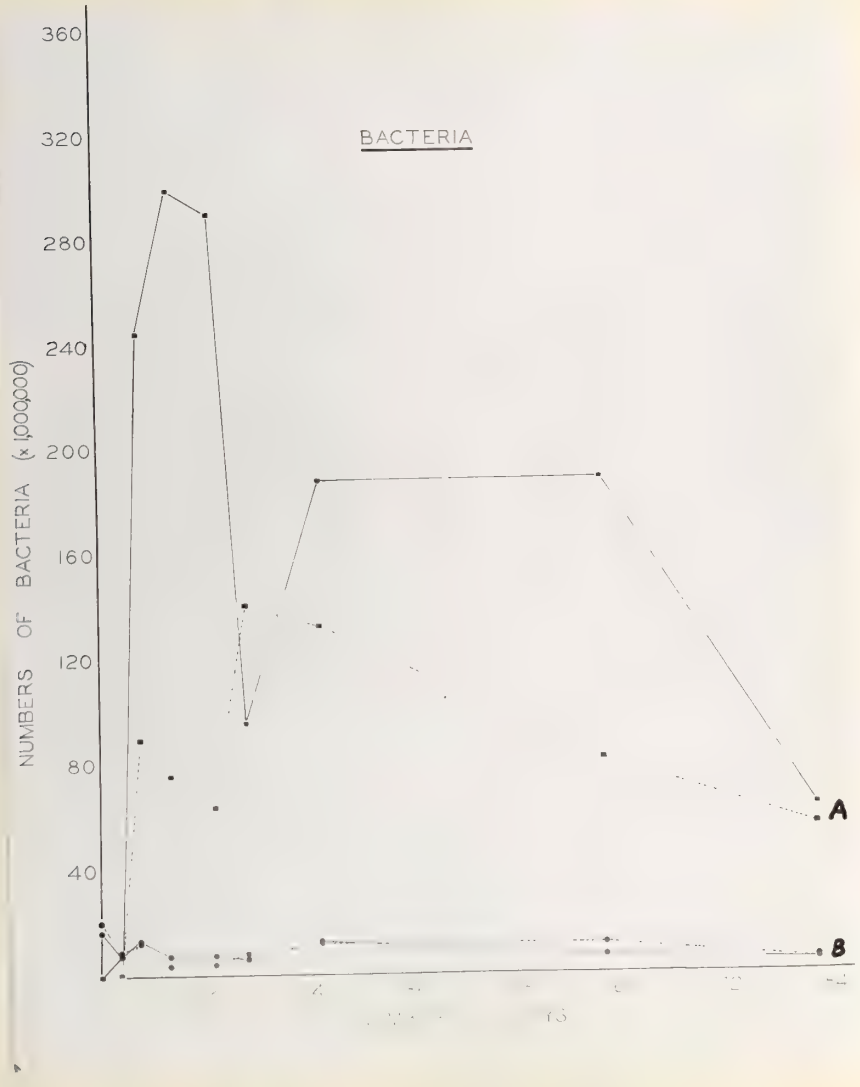
TABLE VI

Comparative numbers of bacteria in sterilized recontaminated and unsterilized soil

No.of days	Sterilized recontaminated		Unsterilized		Average		
	A	A	B	B	Sterilized	Unsterilized	Difference
0	1,703	1,175	16,320,000	20,824,000	1,439	18,572,000	-18,570,561
2	7,469,000	1,738,800	6,440,000	7,920,000	4,603,900	7,180,000	- 2,576,100
4	244,000,000	88,200,000	13,064,000	12,275,000	166,100,000	12,669,500	153,430,500
8	300,000,000	76,000,000	7,260,000	3,600,000	188,000,000	5,430,000	182,570,000
13	291,550,000	64,300,000	7,750,000	5,280,000	177,925,000	6,515,000	171,410,000
17	96,800,000	140,800,000	5,720,000	7,040,000	118,800,000	6,380,000	112,420,000
25	188,650,000	132,000,000	12,100,000	11,592,000	160,325,000	11,846,000	148,479,000
57	189,100,000	82,000,000	7,595,000	11,400,000	135,550,000	9,497,500	126,052,500
80	64,900,000	57,954,000	3,910,000	5,100,000	61,427,000	4,505,000	56,922,000

Figure 3

Comparative numbers of bacteria in sterilized recontaminated and unsterilized soil



A. Sterilized recontaminated soil.

B. Unsterilized soil.

TABLE VII

Comparative numbers of actinomycetes in sterilized recontaminated and unsterilized soil

No.of days	Sterilized recontaminated		Unsterilized		Average		
	A	A	B	B	Sterilized	Unsterilized	Difference
0	104	125	1,360,000	736,000	115	1,048,000	-1,047,885
2	0	0	2,024,000	1,980,000	0	2,002,000	-2,002,000
4	0	0	2,208,000	1,960,000	0	2,084,000	-2,084,000
8	0	2,000,000	2,640,000	2,400,000	1,000,000	2,520,000	-1,520,000
13	0	2,200,000	3,740,000	3,740,000	1,100,000	3,740,000	-2,640,000
17	2,200,000	4,400,000	3,080,000	4,300,000	3,300,000	3,690,000	- 390,000
25	4,900,000	4,400,000	3,520,000	4,990,500	4,650,000	4,255,250	394,750
57	2,200,000	26,000,000	4,860,000	5,400,000	14,100,000	5,130,000	8,970,000
80	7,420,000	13,332,000	1,700,000	4,250,000	10,376,000	2,975,000	7,401,000

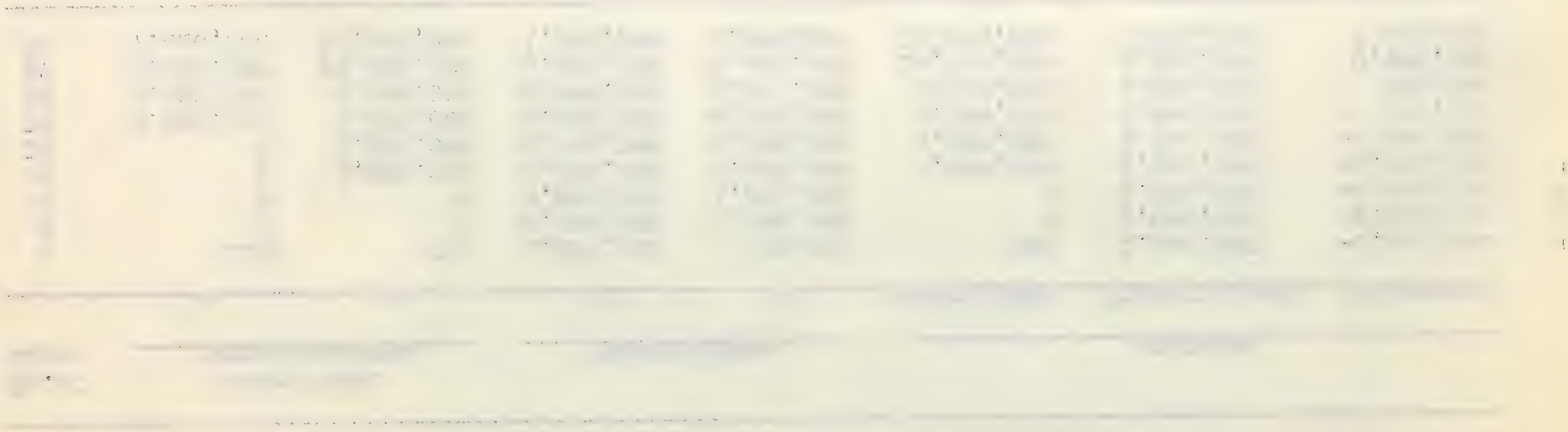
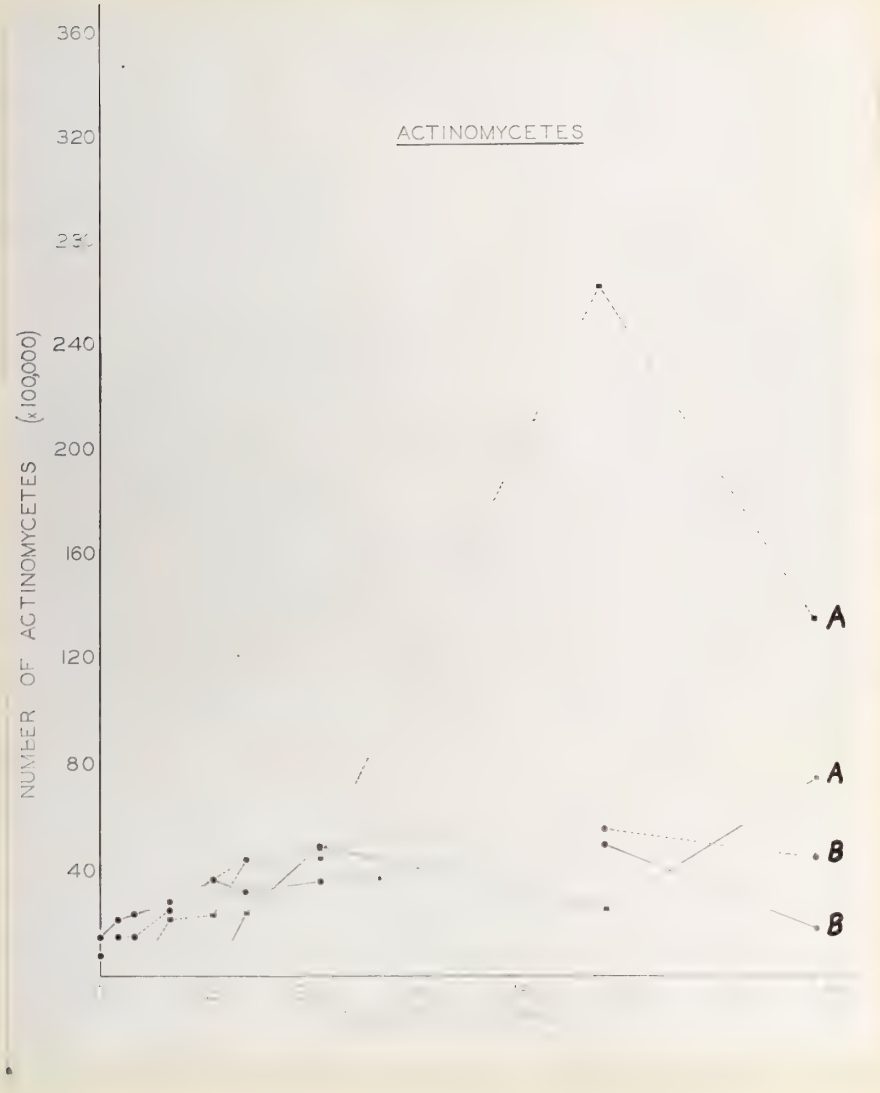


Figure 4

Comparative numbers of actinomycetes in sterilized recontaminated and unsterilized soil



A. Sterilized recontaminated soil.

B. Unsterilized soil.

of the unsterilized soil after a period of 57 to 80 days.

Discussion.

The plate count method is open to criticism on the grounds that only members of the particular group of soil organisms that will grow on the medium chosen are obtained. Further, in the case of the fungi, colonies obtained are largely of single spore origin, and consequently profusely sporing types will appear more often on the plates than those which sporulate poorly or not at all.

No indication, therefore, of the relative abundance of different fungi in the soil is obtained. This objection is particularly valid in a study such as the present one where sterilized soil is involved, since sporulation is more pronounced on this medium. In spite of these objections it was felt that indications of increased growth, in at least some groups of fungi, could be demonstrated. While the relative numbers of different fungi are largely proportional to the spore producing ability of the various types, there is also a great increase in vegetative growth. Profuse mycelial growth in sterilized recontaminated soil can often be observed. The comparatively small number of fungi obtained after an incubation period of two days (Table V, Figure 2) may be attributed to the fact that the fungi have not had sufficient time to produce spores. Large increases in bacterial numbers have been demonstrated. The absence of

actinomycetes in early platings may be attributed to the fact that extremely high dilutions had to be used for bacterial counts, and the slower growing organisms were lost in the dilution.. The indications are, however, that this group is not as important as are the bacteria and fungi. While the group as a whole may not be important, the fact that certain individuals may exert striking antagonistic effects must not be ignored.

The large increase in numbers of microorganisms as well as the increase in activity as measured by increased ammonia production may well be a factor contributing to the inhibition of Ophiobolus graminis. This increase in numbers would result in increased competition for the pathogen. Either the new population as a whole, or certain individuals predominating in the new population, might be responsible for the effect.

Comparative Changes in Kinds of Microorganisms

Introduction.

It has been shown that large increases in numbers of microorganisms follow the recontamination of steam sterilized soil with a little normal soil. In addition to this, it is possible that the kinds of microorganism predominating in recontaminated steam sterilized soil may differ from those in unsterilized soil. This has been

shown to be the case in partially sterilized soil. Russel and Hutchinson (24), for example, observed that, in heated soils, the new flora was different from that in the untreated soil. In toluened soil they found the new flora to be the same as the old. Waksman and Starky (30, 31) recorded that bacterial numbers increased markedly in soil treated with a small amount of toluene, while the number of fungi decreased. The treatment is reported to have made the soil a much better medium for fungi so that those which survived, namely Zygorhynchus, Penicillium, Cunninghamella and Cephalosporium increased in numbers.

As has already been pointed out, however, partially sterilized soil differs from sterilized recontaminated soil in that in the latter case all the organisms originally present are returned after treatment, while in the former only those resistant to the sterilization treatment remain.

Methods.

The results reported here were obtained from the plates used in the quantitative work just reported and from other similar experiments. The results of platings from pot as well as flask culture are included. At the time of making counts most of the colonies were small. These were allowed to develop further, and, in the case of the fungi, to sporulate before the notes as to the kind of organism were made.

Results.

In platings of unsterilized soil the following genera of fungi, arranged in order of decreasing prominence, were obtained with greatest frequency: Penicillium (at least four distinct species), Macrosporium, Aspergillus, Rhizopus and Zygorhynchus. A variety of other fungi such as Trichoderma and Fusarium as well as numerous unidentified types were occasionally obtained. The Penicillia were by far the most prominent.

Early platings of sterilized recontaminated soil yielded mostly Penicillium and Rhizopus. These appeared to be the same as those found in unsterilized soil. Following an incubation period of eight to ten days, however, Trichoderma became the predominating genus. After 80 days, the end of the longest series plated, this was still the case. Once this fungus appeared it became impossible to study other genera, since the plates were rapidly overgrown by it. Trichoderma was almost invariably obtained from platings of incubated pots and flasks even though it only occasionally appeared in platings of unsterilized soil.

No striking differences in the type of bacterial colony were observed. However, in one instance a bacterial colony appeared consistently on the platings from sterilized reinoculated soil and not on those from unsterilized soil. This isolate grew poorly on potato dextrose agar to which it was transferred and was lost. It was never observed in subsequent platings.

No outstanding differences were observed in the character of the actinomycete colonies found on recontaminated sterilized soil as compared with unsterilized soil.

Discussion.

The almost constant appearance of Trichoderma in sterilized recontaminated black soil was demonstrated. A culture of this organism was sent to Dr. Thom of the United States Department of Agriculture and its identification confirmed. Although no reference was made by Dr. Thom to species, it is believed to be T. lignorum. Until further studies are conducted it will be referred to as Trichoderma sp.

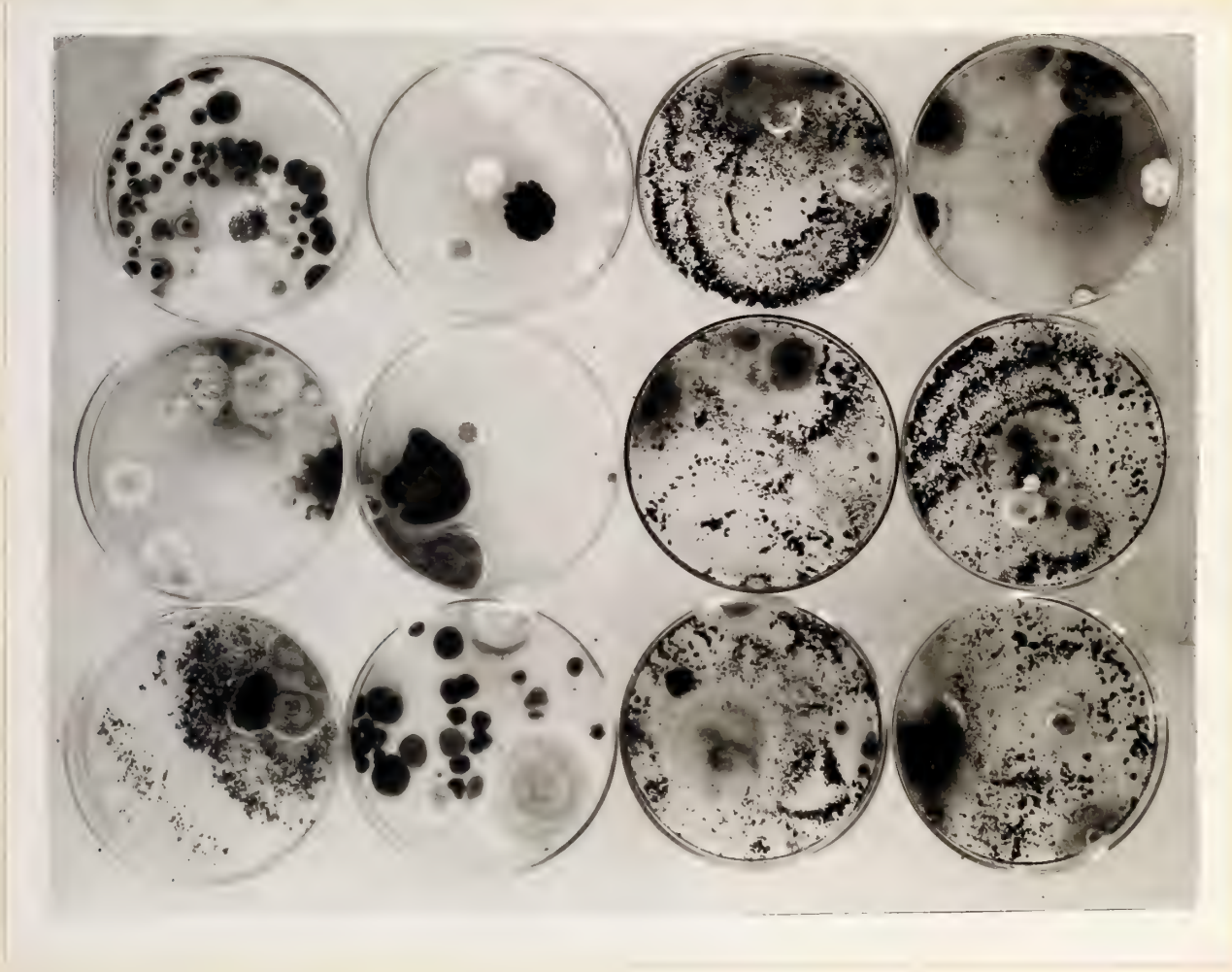
The Growth of Trichoderma sp. in Sterilized and Unsterilized Soil

It has already been noted that a species of Trichoderma almost invariably appeared in platings of sterilized soil which had been recontaminated and incubated for several days. This was not the case in direct platings of unsterilized soil. It was found that, in sterilized soil, an incubation period of 8 to 10 days was necessary before this fungus could be readily isolated by the plating method.

The plates illustrated in Figure 5 deserve special attention. They are representative platings of

Figure 5

Plates showing relative prevalence of Trichoderma in sterilized reinoculated and unsterilized soil



Right - 6 plates from sterilized recontaminated soil showing Trichoderma in all except the one in the upper right hand corner.

Left - 6 plates from unsterilized soil in which Trichoderma only appeared in one plate, that in the lower left hand corner.

soil taken from the pots shown in Figure 1 and reported in Table IV. This plating was done at the conclusion of the experiment and therefore after an incubation period of seven weeks. The Trichoderma rarely failed to appear in the plates from the sterilized recontaminated series, while it was only obtained from one sample of unsterilized soil. The dilution used in plating the sterilized recontaminated soil was ten times as great as that used for the unsterilized soil. The occasional occurrence of Trichoderma in platings of unsterilized soil is to be expected. In this case there was an opportunity for cross contamination from the sterilized reinoculated series to occur.

The frequent failure to isolate Trichoderma from unsterilized soil may be due to its failure to fruit abundantly under these conditions. It has been shown that, in the case of Helminthosporium sativum (20), spore production was inhibited in unsterilized soil, while it occurred freely in sterilized soil. The microbiological nature of the effect is indicated by the fact that small amounts of unsterilized soil added to the sterilized soil results in inhibition of sporulation. A similar effect with a variety of fungi has been noted in this work. One of these, Trichoderma, is shown in Figure 6. When introduced into sterilized soil the rapid growth of this organism as well as its antagonistic effect enables it to predominate even though a great variety of other organisms are present. However, it apparently cannot do so when

Figure 6

Comparative development of *Trichoderma* sp. on sterilized and unsterilized soil

Left - A flask of sterilized soil showing profuse growth and sporulation of *Trichoderma*.

Right - A flask of unsterilized soil on which *Trichoderma* has apparently failed to grow.

introduced into unsterilized soil where the other organisms are already established.

The Occurrence and Distribution of *Trichoderma* on the University of Alberta Campus

There are numerous references in the literature to the occurrence of species of *Trichoderma* in the soil. The only one which will be mentioned at this point is that of Gilman and Abbott(10) who list four species, namely, *T. album*, *T. koningi*, *T. lignorum* and *T. glaucum*. According to Gilman and Abbott these have been found in the soil of the following places:

T. album - United States: Iowa, Louisiana, New Jersey.

T. koningi - England, Holland, Japan, United States: Alaska, California, Hawaii, Iowa, Louisiana, Maine, Michigan, New Jersey, New York, Oregon, Porto Rico, Rhode Island and Utah.

T. lignorum - Canada, England, United States: Iowa, Louisiana, Maine, Oregon, New Jersey, New York, Porto Rico and Utah.

T. glaucum - United States: Iowa.

The predominating species found in this investigation was a green form. With the exception of *T. album* all the above species are green. The species reported here is thought to be *T. lignorum*. A comparison

with a known culture showed it to be very similar. Further identification studies are necessary.

A survey was made to ascertain the general prevalence of Trichoderma species on the University of Alberta campus. The method of isolation employed made use of the fact that this fungus grows exceedingly well in sterilized soil. A number of flasks of soil were made up to a uniform moisture content, sterilized, and then re-contaminated with a sample of soil or dust. Duplicate samples were taken from each location. The flasks were recontaminated at the point of drawing the sample in order to avoid possible contamination in the laboratory. After incubating these flasks of sterilized recontaminated soil for a period of two weeks, dilution plates were poured. A dilution of 1:40,000 was used. The distribution of Trichoderma on the University campus as determined by this limited survey is recorded in Table VIII. As will be seen, the Trichoderma was obtained at all locations, more variation occurring between replicates than between different locations. It may be concluded that the Trichoderma sp. is present in all the soil and dust about the campus.

TABLE VIII

Distribution of *Trichoderma* on the University of Alberta campus

Source of sample	Number of plates out of four showing Trichoderma	
	(a)[2]	(b)
1.[1] Greenhouse - dust beneath bench	4	4
2. Greenhouse - soil beneath bench (2" below surface)	4	4
3. Greenhouse - dust off bench	4	4
4. Soil room - dust	4	4
5. Soil bin - surface dust	4	4
6. Soil bin - 2" beneath soil surface	4	4
7. Sub-basement - dust	4	4
8. Campus field - wheat plots - surface dust	1	3
9. Campus field - wheat plots - 2" below surface	4	0
10. Campus field - summerfallow - surface dust	4	4
11. Campus field - summerfallow - 2" below surface	4	4
12. Investigation field - wheat plots - surface dust	4	4
13. Investigation field - wheat plots - 2" below surface	4	4
14. Investigation field - summerfallow - surface dust	4	4
15. Investigation field - summerfallow - 2" below surface	4	4
16. West field - fescue sod - surface dust	4	4
17. West field - fescue sod - 2" below surface	4	4
18. West field - summerfallow - surface dust	4	4
19. West field - summerfallow - 2" below surface	0	4
20. Road dust collected on 89th Avenue just south of the laboratory	4	4

1 Locations 1 to 7 were in the laboratory and attached greenhouses.

2 (a) and (b) represent the duplicate samples. Four plates were poured from each sample.

THE EFFECT OF INDIVIDUAL ORGANISMS ON THE INFECTION OF WHEAT SEEDLINGS BY *OPHIOBOLUS GRAMINIS* IN STERILIZED SOIL

Introduction

The antibiotic relationships of microorganisms have been comprehensively reviewed by Waksman (29), Garrard and Lochhead (9) and Porter and Carter (23). Only a few of those concerned specifically with the foot-rotting fungi of wheat under Edmonton conditions need therefore be mentioned here. Sanford and Broadfoot (25) tested the effect of a number of organisms and found fungi, bacteria and actinomycetes that reduced the severity of the attack of wheat seedlings by *Ophiobolus graminis*. Their organisms were, however, not strictly soil organisms, but originated from organic material of one kind and another. Henry (12) worked with a group of soil organisms, including fungi, bacteria and actinomycetes, and found them to be antagonistic to *Helminthosporium sativum*. They were, however, less effective in reducing the disease than was the normal soil flora. Individual organisms were not studied.

The main object of this phase of the investigation was to determine whether any particular group of organisms, namely fungi, bacteria or actinomycetes, contained more antagonistic organisms than the other groups. If this

proved to be the case, such knowledge might be of considerable practical value. It might be possible, for example, to encourage the particular group, showing this antagonistic effect, in field soils by appropriate soil management practices.

Methods

The organisms used in these tests were obtained directly from the soil by the plating method. Most of them came from unsterilized soil, but a few such as the Trichoderma sp. were obtained by recontaminating sterilized soil and incubating for a few weeks prior to plating. All tests were made in pot culture, each treatment being replicated five times. Cultures of the saprophytes were, for the most part, grown on a soil-cornmeal mixture, but in the case of a few of the bacteria and actinomycetes water suspensions of cultures from potato dextrose agar were used. The inoculum of the pathogen was invariably grown on a soil-cornmeal medium. Fifty grams of this mixture were used in each flask. In making up the pots half the contents of a flask containing the saprophyte and half the contents of the flask containing the pathogen were added to each pot three-quarters full of sterilized soil. These were mixed with the surface soil of the pots and, after sowing Red Bobs wheat at the rate of 25 seeds per pot, were covered with approximately an inch of sterilized soil.

Where water suspensions were used these were poured on the soil after the pathogen had been added. During the growth of the plants the temperature was maintained at as close to 20°C. as was possible.

Notes on the amount of disease were taken after three to four weeks growth. The size of an individual experiment was limited by the number of flasks available for the production of inoculum.

Results

In a group of three experiments 78 organisms were tested. Of the 20 actinomycete and 16 bacterial cultures included in these tests, none proved to be antagonistic, while of the 42 fungi only four proved to be antagonistic. The effectiveness of these four in suppressing Ophiobolus graminis is indicated by the results presented in Table IX. It is realized that these results hold only for the conditions under which the tests were made.

TABLE IX

The effect of individual soil fungi on the infection of wheat seedlings by Ophiobolus graminis

Culture No.	Identity	Shoot length	Corresponding control
32	Gliocladium[1]	23.7	9.5
1001	Trichoderma	20.3	12.7
1006	Unidentified	21.5	12.7
1013	Unidentified	21.2	12.7

[1] Identified by Dr. Charles Thom, United States Department of Agriculture, Washington, D.C.

These results cannot be compared statistically, since they originate from two separate experiments. Culture No. 1001 is the Trichoderma sp. occurring commonly in sterilized recontaminated soil.

Discussion

It is particularly interesting to note that two of the four antagonistic organisms, obtained from a group of 78 organisms selected at random from soil isolates, should be a Trichoderma sp. and a Gliocladium sp. Particular significance is attached to the former because of the known antagonistic effect of members of this genus on a number of plant pathogens and because of its constant association with sterilized recontaminated soil. The latter is noteworthy because of its consistent antibiotic effect on Ophiobolus, and because members of this genus are mentioned frequently in the literature as being antagonistic to a variety of fungi such as Rhyzoctonia solani and Pythium sp. (34).

THE COMPARATIVE EFFECT OF AN ANTAGONISTIC ORGANISM ON DIFFERENT FOOT-ROTTING PATHOGENS OF WHEAT

Methods

The method used here was the same as that outlined in the preceding section. All inoculum was produced on a soil-cornmeal medium. Three pathogens, Ophiobolus graminis Sacc., Fusarium culmorum and Helminthosporium sativum P.K.B., were used. The saprophyte employed was the Gliocladium sp. reported in the previous section. Pots containing Ophiobolus were held at 18 to 20°, while those with Helminthosporium and Fusarium were held at 22 to 25°C.

Results

The results of this test are given in Table X and illustrated in Figure 7.

TABLE X

The effect of Gliocladium on Ophiobolus, Helminthosporium and Fusarium as measured by the reaction of wheat seedlings to these pathogens

Pathogen	Infection rating in percent		
	Control	Gliocladium	Difference
Ophiobolus	33.8	17.4	-16.4
Control	0.0	--	--
Helminthosporium	65.4	31.4	-34.0
Fusarium	95.3	22.5	-72.8
Control	0.0	--	--

Figure 7

The effect of Gliocladium sp. on Ophiobolus, Helminthosporium and Fusarium as measured by the reaction of wheat seedlings to these pathogens

A B

From left to right - A. Uninoculated control, Ophiobolus alone and Ophiobolus plus 32.

B. Helminthosporium plus 32, Helminthosporium alone, Fusarium plus 32, Fusarium alone and uninoculated control.

Each group represents the plants from a single pot.

The severity of the disease was in all cases markedly reduced.

Discussion

The results show that a single saprophytic organism can, under soil conditions, inhibit all three of the foot-rotting pathogens of wheat. It would seem, therefore, that at least some of the microbiological conditions operating in the decline of *Ophiobolus graminis* in soil may also be effective in bringing about the decline of other foot-rotting pathogens of wheat.

THE EFFECT OF TEMPERATURE ON THE ANTAGONISTIC ACTION OF *TRICHODERMA* SP. AND *GLIOCLADIUM* SP.

Introduction

Two organisms, namely *Trichoderma* sp. (No. 1001) and *Gliocladium* sp. (No. 32), were selected for further study. Henry (14) demonstrated that a greater protective effect was shown in unsterilized soil at high temperatures than at low temperatures, while in sterilized soil (not recontaminated) the disease was equally severe over a range of temperature from 15 to 28°C.

One of the main objects in studying the effect of temperature at this point was to determine if either of these organisms might offer a suitable explanation for the results reported by Henry.

Reaction in Soil

Methods.

The methods used in these experiments were essentially the same as those previously employed. The experiment was conducted in crocks filled with a 3:1 black loam-sand mixture. These were sterilized for 10 hours at 15 pounds steam pressure and allowed to cool in the sterilizer. Inoculum was then added, and wheat was

sown immediately. The fungi were grown on a soil-cornmeal mixture. After removing the surface soil with a sterile tin, half a flask of saprophyte culture, and half a flask of Ophiobolus culture were added. Twenty-five seeds of Red Bobs wheat were then sown in each crock and covered with the original soil. The crocks were now placed in the cans of constant soil temperature tanks. In each tank there were four crocks inoculated with Ophiobolus alone, and four with Ophiobolus plus the saprophytic organism. The four tanks were maintained at water temperatures of approximately 15°, 20°, 25° and 30°C. respectively for the duration of the experiment. A daily record of soil temperature was kept.

Results.

The summarized data for the Gliocladium sp. are presented in Table XI. At the intermediate temperatures the amount of disease was significantly reduced, while at the two extremes, 13° and 28°, the differences were insignificant.

An examination of the data for the Trichoderma sp., Table XII, shows that there was no reduction in disease severity at 15°, but a very marked reduction at 28°C. These reactions are illustrated in Figure 8.

It is of interest to note in passing that Pyronema confluens, Figure 9, a common surface organism on sterilized soil, was almost completely inhibited by the Trichoderma. This was true at all four temperatures used.

TABLE XI

Antagonistic effect of Gliocladium sp. on Ophiobolus at different soil temperatures as measured by the reaction of wheat seedlings to the pathogen

Temperature	% infection rating		Difference
	Gliocladium	Check	
13°C.	19.6	26.3	- 6.7
18°C.	23.0	40.7	-17.7
23°C.	14.3	36.3	-22.0
28°C.	16.4	18.6	- 2.2
Minimum significant difference*	--	--	12.2

* F value exceeding the 1% point.

TABLE XII

Antagonistic effect of Trichoderma on Ophiobolus at different soil temperatures as measured by the reaction of wheat seedlings to the pathogen

Temperature	Trichoderma		Check		Difference	
	Height[1]	% infection rating	Height	% infection rating	Height	% infection rating
15°C.	12.8	85.2	10.7	98.6	2.1	-13.4
20°C.	19.0	79.0	13.2	98.5	5.8	-19.5
24°C.	21.8	84.7	14.6	98.4	7.2	-13.7
28°C.	30.0	48.2	18.9	81.8	11.1	-33.6
Minimum significant difference*	-	-	-	-	2.97	15.3

1 Average height of wheat seedlings in centimeters.

* F values exceeding the 1% point.

Figure 8

Antagonistic effect of Trichoderma on Ophiobolus at different soil temperatures illustrated by the reaction of wheat seedlings

From left to right - 15°, 20°, 24° and 28°C.

Upper row - Ophiobolus alone.
Lower row - Ophiobolus plus Trichoderma.

Figure 9

Inhibition of *Pyronema confluens* by *Trichoderma*

A. Sterilized soil

B. Sterilized soil plus *Trichoderma*

Growth in Culture

The growth-temperature relations of Gliocladium and Trichoderma in culture were determined in order to compare the antagonistic action of each organism with its rate of growth.

Methods.

Petri plates, 9 cm. in diameter, each containing 15 cc. of potato dextrose agar, were used in these studies. A 5 mm. plug of agar cut with a sterile cork borer from a petri plate culture of the fungus to be tested, was placed in the centre of a plate. These plates were then distributed in refrigerators and ovens ranging in temperature from 5°C. to 40°C. Five plates were held at each temperature. The largest diameters of the colonies were then measured at intervals.

Results.

Though complete data for Gliocladium sp. are not presented, it is of interest to note that the optimum temperatures for its growth were found to lie between 20° and 25°C., and that it responded to temperature in much the same way as Ophiobolus graminis.

The growth-temperature relations of the Trichoderma sp. are especially interesting. They are presented in Table XIII. The growth optimum in culture lies in the neighborhood of 30°C. with very rapid growth occurring

over a temperature range of 20° to 34°C. At 30°C. the fungus reached the margins of the plates in two days.

TABLE XIII

Growth of Trichoderma sp. in culture

Temperature	Diameter of colonies in mm.					
	Number of days					
	0	1	2	3	5	7
5°C. ± 1°	5[1]	5	5	5	7	8
9°C. ± 1°	5	5	5	8	14	23
14°C. ± 1°	5	5	14	26	51	85
20°C. ± 1°	5	19	32	78	90[2]	--
25°C. ± 1°	5	31	77	90	--	--
30°C. ± 1°	5	40	90	--	--	--
34°C. ± 1°	5	34	75	90	--	--
40°C. ± 1°	5	7	8	8	8	9

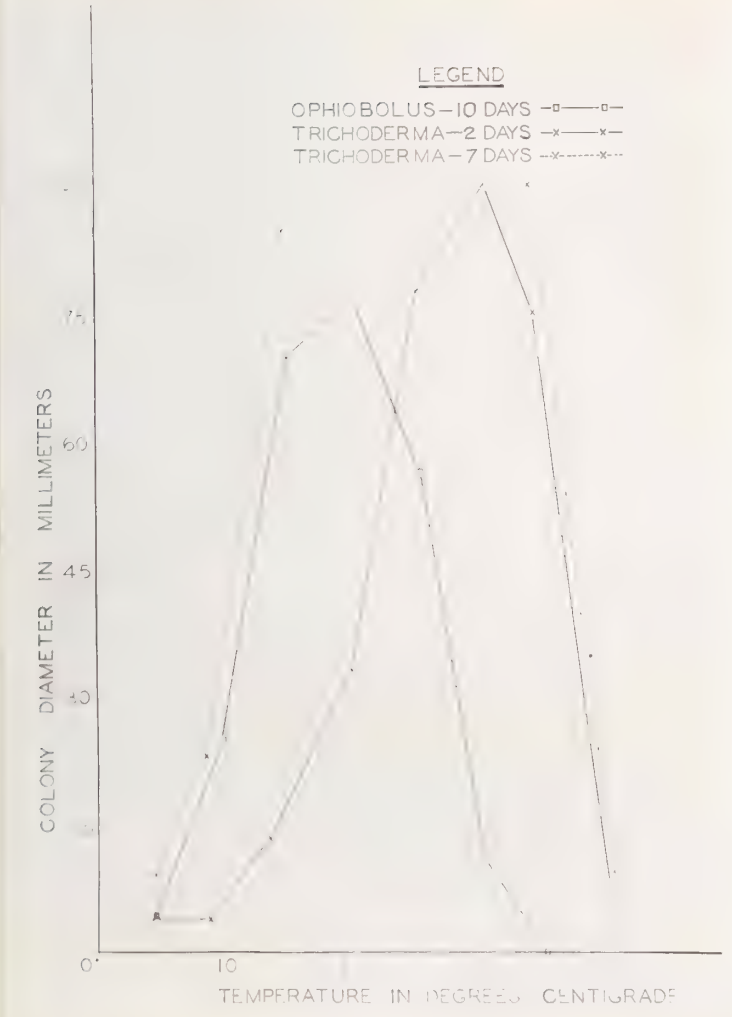
1 Diameter of the block of inoculum in mm.

2 Diameter of the petri plate in mm.

The growth of Trichoderma in 2 and 7 days at the various temperatures is compared graphically with that of Ophiobolus IV, the strain used in these experiments, in Figure 10. The figures for the growth of Ophiobolus on potato dextrose agar were obtained from the work of Davies (4) conducted in this laboratory.

Figure 10

The comparative effect of temperature on the growth of Ophiobolus IV and Trichoderma sp. in culture



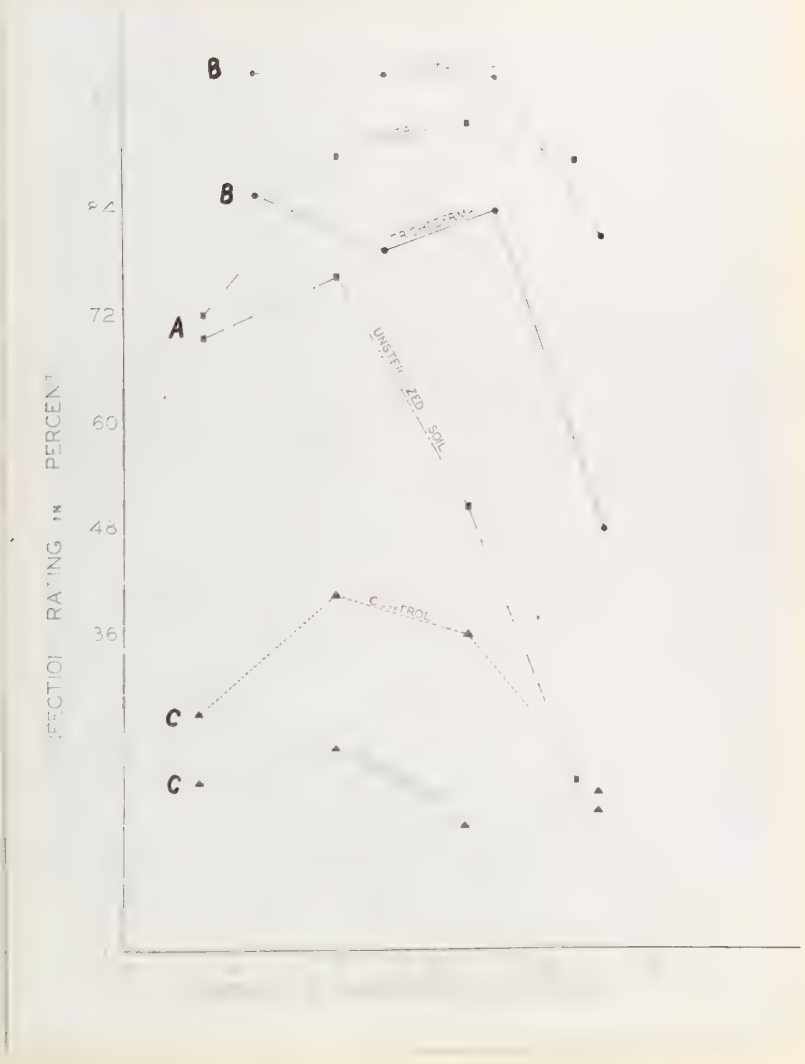
Discussion

Both organisms whose temperature relations were studied appear to be antagonistic to Ophiobolus only over a definite range of temperature. The fact that the Gliocladium is most effective in reducing the severity of "take-all" infection over a temperature range of 18° to 23°C. may account for the consistency of the results obtained with it, since greenhouse temperatures were maintained in the neighborhood of 20° in all these studies. At this temperature consistent antagonistic results with Trichoderma were not obtained. Of the temperatures studied it was most effective at 28°C. Its growth optimum, Figure 10, is in this neighborhood. It is possible that the antibiotic effects of this organism may be partly explainable on the basis of its rapid growth.

The possibility that Trichoderma may be a large contributing factor in the decline of Ophiobolus in natural soils is suggested by a comparison of these results with those of Henry (14). In order to facilitate this comparison the curves for Trichoderma and Gliocladium have been plotted along with those of Henry in Figure 11. It will be noticed that the controls agree closely, differing only in degree of infection obtained. Where Gliocladium was used an altogether different type of curve from that of unsterilized soil was obtained. Trichoderma, on the other hand, gave a curve which corresponds fairly well with unsterilized soil.

Figure 11

The effect of Gliocladium sp., Trichoderma sp. and the combined microorganisms of unsterilized soil on the infection of wheat seedlings by Ophiobolus graminis at different temperatures



A. Unsterilized soil and a sterilized control. Results taken from the work of Henry (14).

B. Trichoderma and the corresponding control.

C. Gliocladium and the corresponding control.

THE RELATION OF SOIL STERILIZATION TO THE PRODUCTION OF ACCESSORY SUBSTANCES

Introduction

The question of whether growth substances exist in the soil is one of great interest. Such substances may be present in natural soil or may be liberated into the soil by heat.

Padwick (21) demonstrated an accessory substance which influenced the growth of Ophiobolus and suggested that such a substance might be present in sterilized soil. Hawker (11) and Asthana and Hawker (1) studied the conditions surrounding reproduction and growth in Melanospora destruens and other fungi. The benefits derived from an accessory substance were especially marked in the reproductive process. Most of the fundamental work on growth substances has, however, been done with the common yeast, Saccharomyces cerevisiae. The early work in the field of yeast growth substances has been reviewed by Tanner (28) and the more recent developments by Bonner (2). A number of growth substances or "bioses" required in minute amounts, have been recognized.

Lilly and Leonian (36) tested a pyridine water extract of unsterilized soil for vitamin B_1 (thiamin) and bios using Phytophthora erythroseptica, Phycomyces blakesleeanus, Pythiomorpha gonapodioides, Mucor

ramannianus and Sordaria fimicola as test organisms. None of the organisms grew on the basic medium alone, but all flourished on the basic medium to which the extract had been added. This suggests the presence of vitamin B_1 and bios in the soil. Further evidence indicates that these may not be entirely associated with living organisms, but may also be present adsorbed to the soil particles. Such a result is not surprising when it is considered that the soil, particularly the more fertile types, contains an abundance of organic material in varying stages of decomposition. In addition, it has been shown that certain organisms, such as Rhizopus nigricans, are capable of manufacturing accessory substances and secreting them into the surrounding medium. It has also been suggested (17), although the writer has been unable to confirm the results, that hormones or some similar substances, which stimulate the growth of the pathogen, Phytophthora cactorum, are excreted by the roots of corn seedlings. The suggestion, therefore, that such substances may be important in soil or may be liberated into the soil on heating is not an unreasonable one.

Methods

Only a very brief outline of the methods used in this phase of the work will be given. Water extracts of soils, using ordinary distilled water, were prepared and sterilized by passage through a Berkfeld filter. Where a

solid medium was used for testing the extracts, a water agar, using washed agar, was made up and diluted with the extract to the desired concentration. Extracts were added after the water agar had cooled to a temperature of about 50°C.

The organisms used for testing the extracts were Ophiobolus graminis, Pyronema confluens and Saccharomyces cerevisiae. Ophiobolus graminis was used because it was the organism with which these studies were especially concerned. Pyronema confluens grows abundantly on the surface of sterilized soil, and consequently it was thought that it might respond well to any materials present in soil extracts. Saccharomyces cerevisiae was used to determine if any or the yeast "bios" fractions were present in soil extracts.

In the yeast work the Toronto strain* of yeast was used. The methods employed were those developed in Miller's laboratory (6, 7) except that 200 cc. erlenmeyer flasks were used in place of rocker tubes. This modification gives lower figures than those recorded by the rocker tube method (6). The procedure followed is indicated in Table XIV. Five cubic centimeters of a salt and sugar solution (S and S) were used in each case. After adding the quantities of other ingredients indicated, the whole was made up to 10 cc. with distilled water so that

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* Obtained through the courtesy of Dr. C. Marchant, University of Toronto.

comparable dilutions would be obtained. Tomato juice was used as a source of "bios I" in order to determine if any toxic materials were present. Counts were made by means of a haemocytometer, a count of one representing 250,000 yeast cells.

Results

No differential results were obtained with Ophiobolus graminis, in either liquid or solid media. Additions of extracts of unsterilized soil to sterilized soil did not result in any inhibition, and conversely additions of sterilized soil extracts to unsterilized soil did not result in growth.

Pyronema confluens grew much better and sporulated more profusely on sterilized soil extract than it did on unsterilized soil extract, although additions of unsterilized soil extract to sterilized soil did not retard the development of the fungus. Growth of this fungus occurred on unsterilized soil when a high humidity was maintained. This growth, however, was very scant, being confined to individual mycelial threads. The occasional fruiting body was also observed.

The growth of yeast on a salt sugar medium, Table XIV, was slightly improved by the addition of sterilized soil extract, an increase of 10 in the count being obtained where the extract alone was added. That

TABLE XIV

The effect of extracts of sterilized and unsterilized soil on the growth of yeast

	Treatment	Count			Average
		1	2	3	
1.	S and S	1.8	1.3	1.0	1.4
2.	S and S + sterilized soil extract 1 cc.	9.8	10.1	-	10.0
3.	S and S + sterilized soil extract 2 cc.	13.7	15.0	14.0	14.2
4.	S and S + sterilized soil extract 1 cc. + tomato juice 1 cc.	323	315	346	328
5.	S and S + sterilized soil extract 2 cc. + tomato juice 1 cc.	287	291	-	288
6.	S and S + unsterilized soil extract 1 cc.	1.0	1.0	2.5	1.5
7.	S and S + unsterilized soil extract 2 cc.	2.3	2.1	1.4	1.9
8.	S and S + unsterilized soil extract 1 cc. + tomato juice 1 cc.	266	261	261	262.6
9.	S and S + unsterilized soil extract 2 cc. + tomato juice 1 cc.	271	246	252	256
10.	S and S + tomato juice 1 cc.	246	261	239	248.6

the material is not "Bios I" of Miller (19) is evidenced by the fact that further additions did not result in quantitative increases in growth and by the fact that addition of "Bios I" in the form of tomato juice resulted in a count of 250. It is possible that one of the other "bioses" was present since the addition of tomato juice (Bios I) plus sterilized soil extract increased the count more than did the extract alone. The unsterilized soil extracts exerted neither a toxic nor a stimulating effect.

Discussion

No evidence of a growth factor for Ophiobolus graminis was obtained and while, in the case of Pyronema confluens, the growth was better in sterilized soil extract, evidence is not sufficient to warrant the conclusion that this is due to an accessory factor rather than to the increased fertility level (24). Indications that one of the secondary growth factors for yeast was present in the water extracts of sterilized soil were obtained. These indications will be followed up as soon as equipment is available.

While most of the evidence in this phase of the work is in disagreement with the growth factor hypothesis, it is not strong enough to warrant any definite conclusions. There is the possibility, for example, that if growth substances are present, they may be strongly adsorbed to the organic matter in the soil and resistant to extraction methods such as were used in these experiments.

GENERAL DISCUSSION

It has been shown by Henry (12, 13, 14) that the microorganisms of unsterilized soil have a "protective effect" against the infection of wheat seedlings by foot-rotting fungi.

In this investigation it was demonstrated that, when sterilized soil is recontaminated with a little normal soil, the suppression of Ophiobolus graminis is greater than that in the natural unsterilized soil. This result confirms earlier findings in this laboratory. A comparative study of the microbiological relations in sterilized recontaminated and unsterilized soil was undertaken in an attempt to explain this phenomenon. It is hoped, from such a study, to obtain a better understanding of the factors responsible for the decline of Ophiobolus in sterilized recontaminated soil, so that it may later be possible to reproduce them under field conditions by appropriate soil management practices.

A study of the numbers of fungi, bacteria and actinomycetes in sterilized recontaminated soil show them, with the possible exception of the actinomycetes, to be greatly increased over the numbers in unsterilized soil. Accompanying this increase in numbers is an increase in activity as indicated by ammonia production (18). Evidence on numbers immediately following recontamination indicates that the actinomycetes as a

group are not the main antagonistic organisms, although it is known (25) that certain individuals exhibit striking antibiotic relationships with Ophiobolus graminis. It is conceivable that this increase in numbers of microorganisms might be detrimental to Ophiobolus in several ways. There might, for example, be an exhaustion of some food material essential for the pathogen. A second possibility is that the rapid growth of the organisms might result in the accumulation of materials toxic to Ophiobolus. Still a third possibility is that the sterilization and recontamination may result in the encouragement of certain individuals which are antagonistic to Ophiobolus. It must also be borne in mind that a number of effects such as these may be, and probably are, operating together in the soil, and that possibly it is their combined effect that is manifesting itself.

The most striking change in the flora of the soil resulting from steam sterilization and recontamination noted was the marked multiplication of a species of Trichoderma. This fungus was almost invariably obtained from sterilized recontaminated soil, but only occasionally from unsterilized soil by direct isolation methods. This organism, having a growth optimum in the neighborhood of 30°C., proved to be more antagonistic to Ophiobolus at temperatures of 24° and 28°C. than at lower temperatures. A significant reduction in the amount of "take-all" has been obtained at 18° to 20°C., although results in repeated experiments at this temperature did not agree.

The Trichoderma sp. encountered in these studies is regarded as being highly significant; firstly, because of its constant association with sterilized recontaminated soil; secondly, because of its wide distribution in nature; thirdly, because of its antagonistic action; fourthly, because members of this genus have been shown to be antagonistic to a number of plant pathogens especially Pythium and Rhizoctonia; and fifthly, because results with it show a striking parallel with those in unsterilized soil. The possibility, therefore, that this organism is one of the major factors responsible for the decline of Ophiobolus in natural soils cannot be ignored.

If we assume that the Trichoderma sp. is responsible for the decrease in disease in sterilized recontaminated soil as compared to unsterilized soils, an explanation of some of the irregular results obtained in these experiments is offered. The temperature, for example, was maintained at 18° to 20°C. during the majority of the experiments. This is definitely below the optimum for growth and for antagonistic action of Trichoderma. Moisture may also be a factor of importance. Other environmental factors such as soil aeration and soil humidity may also vary greatly. Studies on the physiology of the Trichoderma are necessary before further conclusions can be drawn.

The results with Trichoderma suggest one of the difficulties involved in the use of sterilized soil as a medium in which to study soil-borne plant pathogens.

Trichoderma grows rapidly and sporulates profusely in sterilized soil and, as a consequence, in any experiment involving the use of sterilized soil it is difficult to prevent its introduction as a contaminant.

Tests of the antagonistic action on Ophiobolus graminis were made for 78 single organisms, isolated at random from the soil. These consisted of fungi, bacteria and actinomycetes. Under the conditions of the experiments only four fungi were antagonistic to Ophiobolus. One of these was the Trichoderma sp., another a Gliocladium sp., while the remaining two were unidentified. The Gliocladium sp. was the most outstandingly antagonistic organism obtained in these studies, being similar in action to the Gliocladium of Weindling (34). However, studies made so far do not indicate that this organism plays a major part in the suppression of pathogenic fungi and thus in the reduction of disease under natural conditions, since it was only isolated once even though constant watch was kept for it. This was true in spite of the fact that it grows well on artificial media, and that it tends to sporulate abundantly in sterilized soil. Results obtained with it do not agree with those obtained in unsterilized soil in that this organism was most effective in reducing "take-all" at temperatures of 18° and 23°C. and had no significant effect at 28°C. It should be pointed out, however, that the Gliocladium was also ineffective in controlling Ophiobolus at a temperature of 14°C., while at this temperature "take-all" is particularly severe in

unsterilized soil.

No indication of a growth substance for Ophiobolus was obtained in extracts of sterilized soil, although such a material might be held in the soil in a manner preventing extraction by the procedure employed. That such a substance is not directly responsible for the difference in disease occurring in sterilized and unsterilized soil is shown by the still further "take-all" reduction in sterilized recontaminated soil as compared with unsterilized soil. As has been previously pointed out, however, such an effect may be operating in an indirect way.

SUMMARY

1. The results of previous experiments, indicating that less "take-all" infection of wheat occurs in sterilized recontaminated soil than in normal unsterilized soil, were confirmed.

2. In general, the number of microorganisms, as determined by the plate count method, was found to be much greater in sterilized recontaminated soil than in unsterilized soil.

3. The numbers of fungi and bacteria in sterilized recontaminated soil rose rapidly, reached a maximum, and then declined rapidly. After a period of 80 days the numbers were still many times higher than in unsterilized soil.

4. The number of actinomycetes did not increase rapidly in sterilized recontaminated soil. After a period of 80 days, however, the numbers in sterilized recontaminated soil equalled, if not exceeded, the numbers in unsterilized soil.

5. A species of Trichoderma, resembling T. lignorum, was almost invariably obtained from platings of sterilized recontaminated soil, and only occasionally from platings of unsterilized soil.

6. A similar *Trichoderma* was found to be widely distributed in soil and dust samples on the University of Alberta campus.

7. Only four fungi out of 78 soil microorganisms tested, proved to be antagonistic to *Ophiobolus graminis* in soil culture. These included *Gliocladium* sp., No. 32, and *Trichoderma* sp., No. 1001. The former was isolated only once, while the latter was frequently obtained, especially from sterilized recontaminated soil.

8. *Gliocladium* sp., No. 32, was antagonistic to *Fusarium culmorum* and *Helminthosporium sativum* as well as to *Ophiobolus graminis*.

9. *Gliocladium* sp., No. 32, was antagonistic to *Ophiobolus graminis* at soil temperatures of 18°C. and 23°C., but not at 13°C. or 28°C.

10. In soil tests, *Trichoderma* sp., No. 1001, was most antagonistic to *Ophiobolus graminis* at high temperatures, for example, 28°C., and least antagonistic at low temperatures, for example 15°C.

11. The temperatures most favourable for this antagonistic action were roughly those most favourable for the growth of *Trichoderma*, No. 1001, in artificial culture.

12. The reaction of Ophiobolus graminis to Trichoderma corresponded approximately with its reaction to the combined microorganisms of unsterilized black soil.

13. No indications of the presence, in either sterilized or unsterilized soil, of a growth substance for Ophiobolus graminis, were obtained.

14. Some evidence, indicating that a yeast bios differing from "Bios I" may be present in water extracts of sterilized black soil, was obtained. Such a substance was not detected in unsterilized soil.

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APPENDIX I

The comparative effect of unsterilized and sterilized reinoculated soil on the infection of wheat seedlings by *Ophiobolus graminis* with particular reference to the addition of different amendments

Fertilization treatment	Inoculation treatment	Sterilization treatment								
		Sterilized			Unsterilized			Difference		
		Height	Dry weight	% disease	Height	Dry weight	% disease	Height	Dry weight	% disease
16-20	Control	36.0[1]	1.00[2]	0[3]	35.7	1.23	1.1	0.3	-0.23	- 1.1
	Inoculated	27.5	0.97	50.5	20.8	0.74	67.7	6.7	0.23	-17.2
Alfalfa meal	Control	35.3	1.00	0.2	33.0	1.14	0	2.3	-0.14	0.2
	Inoculated	24.8	0.76	52.8	26.2	0.79	52.8	-1.4	-0.03	0.0
Alfalfa meal + 16-20	Control	33.4	0.94	0.6	35.1	1.10	2.1	-1.7	-0.16	- 1.5
	Inoculated	26.4	0.75	48.9	25.8	0.72	46.2	0.6	0.03	2.7
Straw meal	Control	38.0	1.18	0	33.5	1.02	0.6	4.5	0.16	- 0.6
	Inoculated	33.4	1.14	23.1	28.2	0.79	43.6	5.2	0.35	-20.5
Straw meal + 16-20	Control	36.6	1.38	0.2	36.8	1.24	0.4	-0.2	0.14	- 0.2
	Inoculated	34.1	1.14	23.7	29.3	0.79	37.7	4.8	0.35	-14.0
Sawdust	Control	36.9	1.13	0.2	32.9	0.93	1.0	4.0	0.20	- 0.8
	Inoculated	34.2	1.06	19.3	22.4	0.69	55.6	11.8	0.37	-36.3

APPENDIX I (Continued)

Fertilization treatment	Inoculation treatment	Sterilization treatment								
		Sterilized			Unsterilized			Difference		
		Height	Dry weight	% disease	Height	Dry weight	% disease	Height	Dry weight	% disease
Sawdust + 16-20	Control	37.7	1.26	0	34.3	1.10	0.2	3.4	0.16	- 0.2
	Inoculated	33.0	1.01	21.1	28.6	0.88	36.9	4.4	0.13	-15.8
None	Control	35.8	1.34	0	32.5	1.08	0.8	3.3	0.26	- 0.8
	Inoculated	24.9	0.85	52.9	16.7	0.60	81.0	8.2	0.25	-28.1
Fertilizer averages	Control	36.2	1.15	0.2	34.2	1.10	0.8	2.0	0.05	- 0.6
	Inoculated	29.8	0.96	36.5	24.5	0.75	52.7	5.3	0.21	-16.2
Minimum significant difference*		3.29	0.140	--	3.29	0.140	--	3.29	0.140	--

* Minimum significant difference for inoculations.

Minimum significant difference between any two individuals (a) Height = 9.33
(b) Weight = 0.39

1 Plant height in centimeters.

2 Dry weight in grams.

3 Disease rating in percent.

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